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## A CONSTRICTED TUBE WITH MECHANICAL SEAL FOR ANAEROBIC FERMENTATION TESTS

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No attempt is made to chronicle the various devices invented to meet the peculiar requirement of reduced oxygen tension in anaerobic fermentation tests. I was more interested in a method which would reduce that difficulty to a minimum and at the same time contribute to a proper understanding of the test itself. After trying mercury as a seal in the Smith fermentation tube, which fails on account of its germicidal action, and sand, which works but is troublesome, it was decided to combine the principle of the constricted tube (Hall,<sup>1</sup> 1915) with that of the fermentation tube so as to use a mechanical seal.

Since the paper in which the constricted tube was first described did not have a wide circulation, an illustration which appeared therein is reproduced here (Fig. 1) in order to show several types of seal that may be used. Glass, imitation agate, marbles are best, but we have also used a porcelain biconvex disk manufactured by the Star Porcelain Co. of Trenton, N. J., for this purpose, as well as circular cover slips. The two latter have the advantage of permitting the passage of a pipet into the lower portion of the tube, but the seal is less perfect than that of the marble. The mechanical seal principle has been used in flasks also, as shown in fig. 2.

The use of the mechanical seal principle in a fermentation tube is represented in fig. 3. The first lot of tubes was made for the writer at the University of Chicago in 1920. These first tubes were designed with the anaerobic arm of the tube closed, but difficulty in cleaning and a desire to obtain culture material from the closed branch without admixture with that portion of medium exposed to the air above the seal, suggested that the anaerobic arm be closed with a rubber stopper as indicated. The dimensions used are: diameter of open branch 25 mm., diameter of closed branch 9 mm., length 145 mm. Several lots recently made by Mr. W. J. Cummings, glass-blower in the Department of Chemistry at the University of California, according to these

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<sup>1</sup> University of California Publications in Pathology, 1915, 2, p. 147.

specifications have cost \$30 per 100. The tubes have proved to be somewhat fragile, especially if care is not exercised when inserting the rubber stopper to hold the tube by the small branch, but broken tubes are readily repaired by a competent glass-blower at slight expense. Some difficulty was encountered in securing rubber stoppers to fit the closed branch, as a size smaller than those regularly listed was required;

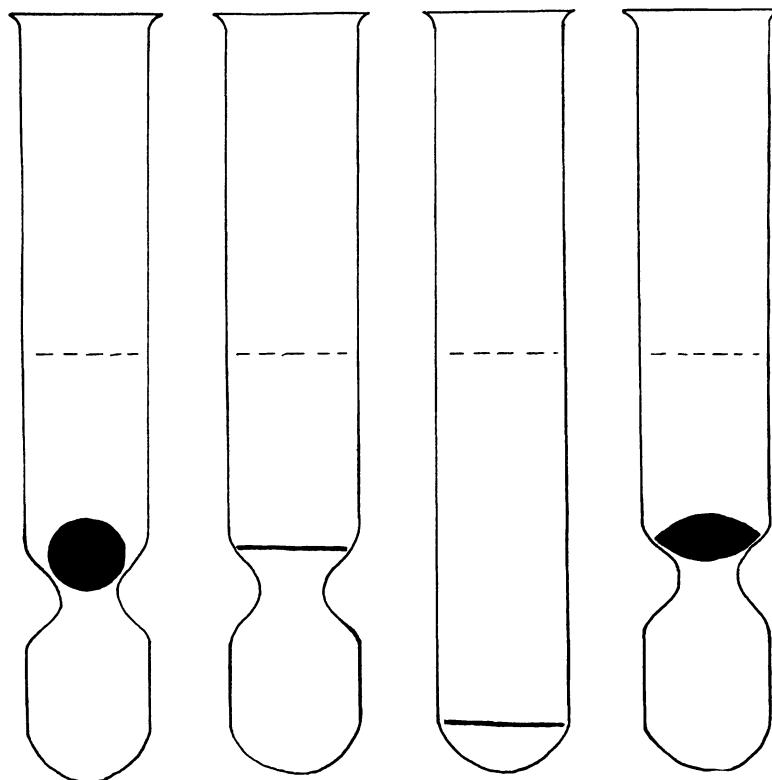


Fig. 1.—Mechanical seals for anaerobic cultures; (a) marble seal in constricted tube; (b) cover slip seal in constricted tube; (c) cover slip in plain tube; (d) biconvex disk in constricted tube. The dotted line represents the height of liquid medium in each tube.

we are now using a good grade of red gum stopper especially manufactured for the Cutter Biological Laboratories of Berkeley, and kindly supplied by them to us. The glass imitation agate marbles used vary in diameter from 15 to 20 mm. The tubes are best plugged for use with a cotton plug covered with surgeon's gauze.

The use of this device is by no means limited to fermentation reactions. It provides a valuable means of supplying culture material from the closed branch for motility tests with a minimum of exposure to air. Such tubes also may serve, with or without the seal, every purpose that the original Smith fermentation tubes serve. Were it not

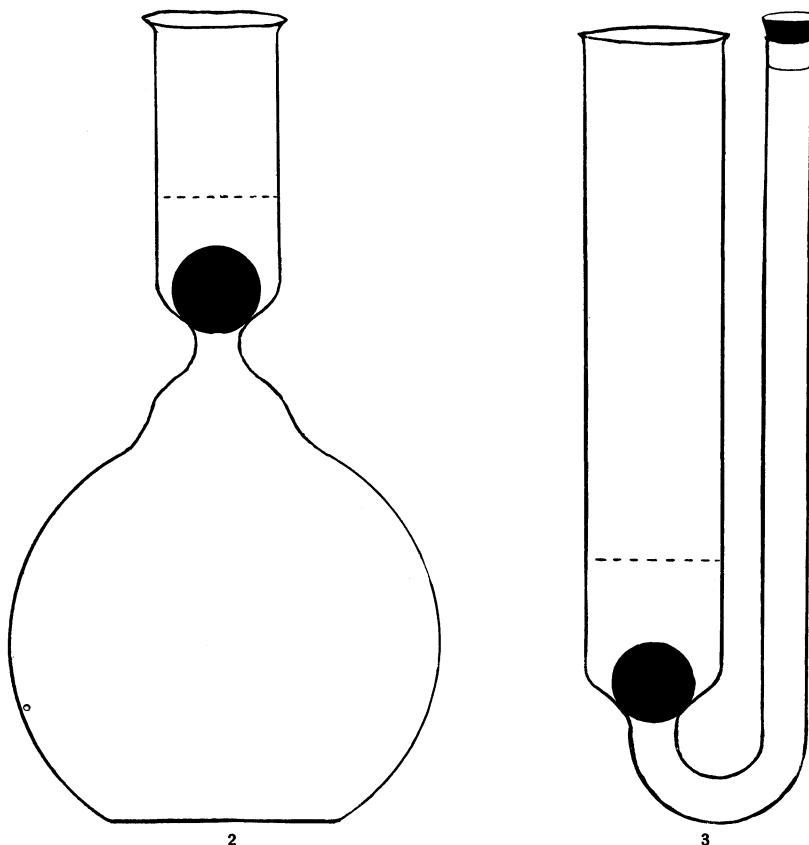


Fig. 2.—Constricted neck flask with marble seal. The dotted line represents the height of liquid medium in the flask.

Fig. 3.—Fermentation tube with marble seal. The dotted line represents the height of liquid medium in the tube.

for the greater expense, the constricted fermentation tube might serve every purpose that the ordinary constricted tube serves, aerobic and anaerobic sterility tests, virulence tests, preparation of immunizing and agglutinating antigens, and all the varied purposes for which a small volume of broth culture of any obligate anaerobe may be desired.

But the principal reason for the invention of this fermentation tube was that an adequate study might be made of the problem of utilizing the fermentation tests of obligate anaerobes in a differential and taxonomic sense, having in mind the fact that peculiar obstacles are encountered in the proper interpretation of the usual criteria of fermentation when applied to these organisms.